



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

**SUBJECT:** Science Review in Support of a Label Amendment and a Petition (Petition No. 0F06144) for an Exemption From the Requirements of Tolerances for EthylBloc™ (EPA Reg. No. 071297-1) Containing 0.14% 1-Methylcyclopropene (Chemical No. 224459). Review of Acute and Subchronic Toxicity Studies and Other Human Health Data/Information. DP Barcode D275229; Case No. 063215; Submission No. S597276; MRIDs 453803-01 to -08

**FROM:** Russell S. Jones, Ph.D., Biologist  
Biochemical Pesticides Branch  
Biopesticides & Pollution Prevention Division (7511C)

**THRU:** Freshteh Toghol, Ph.D., Senior Scientist  
Biochemical Pesticides Branch  
Biopesticides & Pollution Prevention Division (7511C)

**TO:** Driss Benmhend, Regulatory Action Leader  
Biochemical Pesticides Branch  
Biopesticides & Pollution Prevention Division (7511C)

ACTION REQUESTED

AgroFresh, Inc. [formerly BioTechnologies for Horticulture, Inc (BTH, Inc.; a subsidiary of Rohm and Haas Company)] has submitted a new acute and subchronic toxicity studies and other human health data/information in support of: (i) a petition (Petition No. 0F06144) for an exemption from the requirements of tolerances for residues of 1-MCP on stored food commodities; and (ii) a label amendment to add indoor use on post-harvested fruits and vegetables. The new studies consist of a new acute inhalation study, a new battery of mutagenicity studies, a subchronic rat inhalation study, preliminary radiolabeled residue data, a dietary and worker risk assessment, and a new acute oral LD<sub>50</sub> study.

1-MCP is the active ingredient in the end-use product, EthylBloc™ which contains 0.14% 1-MCP. EthylBloc™ is a powdered product that releases 1-MCP as a gas when mixed with water or a buffering agent. The end-use product is currently registered for non-food use on floral and nursery crops.

## DATA FOR ENTRY INTO ISIS

### Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Conc. range ppm	Concentrations ppm	NOAEL ppm	LOAEL ppm	Target organ	Comments
224459	45458608	developmental	rats	GD 6-19	inhal.	whole-body	100-1000	0, 100, 300, 1000	100	300	spleen, kidney, body weight	Maternal
224459	45458608	developmental	rats	GD 6-19	inhal.	whole-body	100-1000	0, 100, 300, 1000	≥ 1000	unknown	none	Developmental

## DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: BACTERIAL REVERSE MUTATION ASSAY  
WITH *SALMONELLA TYPHIMURIUM*  
(AMES TEST)

Prepared for

Biopesticides and Pollution Prevention Division  
Office of Pesticides Programs  
U.S. Environmental Protection Agency  
Crystal Station I  
2800 Jefferson Davis Highway  
Arlington, VA 22202

Prepared by

Tetrahedron, Inc.  
1414 Key Highway  
Baltimore, MD 21230

Primary Reviewer:  
Steven T. Cragg, PhD, DABT

Signature: Steven T. Cragg  
Date: 3 July 2001

Secondary Reviewer  
Nasin Begin, PhD

Signature: Nasin Begin  
Date: 7/3/01

Quality Assurance:  
Waqi Alum, PhD.

Signature: Waqi Alum  
Date: July 3, 2001

EPA Reviewer: Biochemists  
Review Section, Toxicology Branch (7500C)  
EPA Secondary Reviewer:  
Review Section, Toxicology Branch (7500C)  
Biochemists

Quinn B. Jan, Date 1/29/2002

Data Evaluation Record

STUDY TYPE: Bacterial Reverse Mutation Assay with *Salmonella typhimurium* (i.e., Ames Test)  
OPPTS 870.5100 [§84-2]; OECD Guideline 471 [EEC Directive 92/69/EEC B.2]

DP BARCODE: D275229  
P.C. CODE: 071297-00001  
MRID No.: 453803-02

SUBMISSION CODE: S597276  
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: O'Neill, P.J., Frederick, C.B., (2001). 1-Methylcyclopropene Vapor Released from 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.): *Salmonella typhimurium*. Gene Mutation Assay. Rohm and Haas Company Toxicology Department Report No. 00RC-193A, February 27, 2001. MRID 453803-02. Unpublished.

SPONSOR: Rohm and Haas Company  
Toxicology Department  
727 Norristown Road  
Spring House, PA 19477-0904

**EXECUTIVE SUMMARY:** In a reverse mutation assay in bacteria, (MRID 453803-02), strains TA 98, TA100, TA102, TA1535, and TA1537 of *S. Typhimurium* were exposed for 24 hours to atmospheres containing 1-methylcyclopropene (1-MCP) released from a 1-MCP/alpha-cyclodextrin complex (a.i. 3.3%) at nominal concentrations of 0, 100, 300, or 1000 ppm 1-MCP in an initial assay and 0, 100, 300, or 1000 ppm in a confirmatory assay. Concentrations were analyzed by gas chromatography at three time points (1, 4, and 24 hr) over a 24-hour exposure period. After exposure to 1-MCP, cultures were incubated for a further 24 hours. Tester strains were exposed to 1-MCP in the presence and absence of a mammalian metabolic activation system. The activation system consisted of the post-mitochondrial centrifugation (S-9) fraction of liver from rats treated with the P-450 enzyme inducer, Aroclor 1254.

1-MCP was not tested at higher concentrations than 1000 ppm because, according to the authors, higher concentrations might result in an explosion hazard. No cytotoxicity was reported at any test concentration. No increase in revertant frequencies was detected in either the initial or confirmatory assays in any of the 5 tester strains with or without S-9 metabolic activation. An exception was for Strain TA1535 at 1000 ppm only in the confirmatory test (with activation), where revertant frequencies were exactly two times background. Because the background was unusually low by historical standards, a repeat confirmatory assay was performed with only TA1535. This repeat confirmatory test was negative. Chemical analysis of exposure atmospheres indicated that actual concentrations ranged from 78% to 126% of target concentrations. The strains reacted appropriately to positive control chemicals, yielding revertant frequencies within expected ranges. The results of this study indicate that 1-methylcyclopropene is not mutagenic in this test system.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for *in vitro* mutagenicity (bacterial reverse mutation) data and was conducted according the EPA OPPTS 870.5100 protocol for this assay.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

## I. MATERIALS AND METHODS

### A Materials:

#### 1. Test Material: 1-Methylcyclopropene

Description: Colorless gas released from *alpha*-cyclodextrin matrix (3.3% a.i.) (white solid with business confidential composition included as Confidential Attachment).

Lot/Batch #: Lot No. BAS 5-80.

Purity: 3.3% within matrix; varying percentages when allowed to vaporize from matrix in Tedlar exposure bags upon addition of water.

CAS #: 3100-04-7 (a.i.).

Solvent used: None (cells were exposed directly to 1-MCP vapor).

Other comments: 1-MCP is released from the *alpha*-cyclodextrin matrix when mixed with warm water.

#### 2. Control Materials:

Negative:  
*alpha*-Cyclodextrin powder without 1-methylcyclopropene (with water added to simulate releasing conditions)

#### Positive:

##### Nonactivation:

1-Nitrofluorene	50	µg/plate	TA98
Sodium azide	2	µg/plate	TA100, TA 1535
9-Aminoacridine	100	µg/plate	TA1537
Mitomycin-C	2	µg/plate	TA102

##### Activation

2-Anthramine	5	µg/plate	TA98, TA100, TA1535
2-Anthramine	6	µg/plate	TA1537
2-Anthramine	12	µg/plate	TA102

#### 3. Activation: S9 derived from

<input checked="" type="checkbox"/> Aroclor 1254	<input checked="" type="checkbox"/> Induced	<input checked="" type="checkbox"/> Rat	<input checked="" type="checkbox"/> Liver
<input type="checkbox"/> Phenobarbital	<input type="checkbox"/> Non-induced	<input type="checkbox"/> Mouse	<input type="checkbox"/> Lung
<input type="checkbox"/> None		<input type="checkbox"/> Hamster	<input type="checkbox"/> Other
<input type="checkbox"/> Other			<input type="checkbox"/> Other

S-9 mix was purchased from Molecular Toxicology, Inc. [Moltox], Lot No. 955 and 1111, and consisted of the following:

<u>Component</u>	<u>Final Concentration</u>
Nicotinamide-adenine dinucleotide phosphate (NADP)	4 mM
Glucose-6-phosphate	5 mM
Magnesium chloride (MgCl <sub>2</sub> )	8 mM
Potassium chloride (KCl)	33 mM
Sodium phosphate buffer, pH 7.4	100 mM
Liver homogenate (S-9) from Aroclor 1254 treated rats	10 µl/ml

4. Test Cells: *S. typhimurium* strains (obtained from B. Ames, U California, Berkeley)

☐ TA97    ☒ TA98    ☒ TA100    ☒ TA102    ☒ TA1535  
☒ TA1537    ☐ TA1538

Properly maintained? Y

Periodically checked for Mycoplasma contamination? Y

Periodically checked for karyotype stability? Y

Periodically "cleansed" against high spontaneous background? Y

5. Test compound atmospheric concentrations used

"Initial Definitive" assay:

Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)

Activated conditions: 0, 100, 300, 1000 ppm (nominal)

"Confirmatory" assay:

Nonactivated conditions: 0, 10, 50, 500, 1000 ppm (nominal)

Activated conditions: 0, 10, 50, 500, 1000 ppm (nominal)

"Repeat Confirmatory" assay (with strain TA 1535 only):

Nonactivated conditions: 0, 10, 50, 500, 1000 ppm (nominal)

Activated conditions: 0, 10, 50, 500, 1000 ppm (nominal)

All strains (triplicate plates) were used for each treatment condition.

B. TEST PERFORMANCE

1. Type of Salmonella assay

☒ Standard plate test  
☐ Pre-incubation test  
☐ "Prival" modification (i.e., azo-reduction method)  
☐ Spot test  
☐ Other (describe)

2. Protocol:

To achieve the desired atmospheric concentration, 1-MCP was released from a quantity of alpha-cyclodextrin matrix selected to achieve a predetermined concentration inside a 12 liter Tedlar air-tight bag. 1-MCP was released when the *alpha*-cyclodextrin matrix containing it was dissolved with warm water inside the bag. Minimal agar dishes (in triplicate) overlaid with the five bacterial strains (with and without metabolic activation systems) were placed inside the bags prior to release of 1-MCP. Once water was mixed with the 1-MCP-containing *alpha*-cyclodextrin matrix, bags were sealed and placed into incubators at 37°C for 24 hours. The atmospheres inside the bags were sampled at 1, 4 and 24 hours by gas chromatography (with a flame ionization detector). After 24 hours, plates were removed from the bags, lids were added, and plates were returned to the incubator for an additional 24 hours. Colonies were counted at the end of at least 48 hours incubation. To achieve a positive response, revertant counts in strains exposed to the test material had to exceed negative control counts by a factor of two. Toxicity was defined as the elimination of a uniform background lawn.

## II. REPORTED RESULTS

### A. Preliminary cytotoxicity assay

No results of a preliminary cytotoxicity assay were reported. Although the criteria for judging cytotoxicity (i.e., elimination or diminution of uniform background lawn) was stated in the "methods" section describing the definitive and confirmatory assays, the issue of cytotoxicity was not addressed in the results section of the report. Nevertheless, the fact that revertant colonies for 1-MCP-exposed plates were approximately the same as for negative controls indicates a lack of toxicity, even at the highest exposure level tested.

### B. Definitive assay (0, 100, 300, and 1000 ppm)

Revertant counts did not exceed the negative control for any of the five tester strains at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See Table 1 for mean summary revertant counts.

### C. Confirmatory assay (0, 10, 50, 500, 1000 ppm)

For four of the five tester strains, revertant counts did not exceed the negative control at any concentration, with or without metabolic activation. For tester strain TA 1535, revertant counts exceeded negative control counts by a factor of exactly 2 at 1000 ppm only. Because the negative control was considered low by historical standards, it was suspected that this result was spurious. As a result, the test was repeated using only this strain (see below). Positive control revertant counts were within expected ranges for all strains. See Table 2 for mean summary revertant counts.

### D. Repeat Confirmatory assay with TA 1535 only (0, 10, 50, 500, 1000 ppm)

When retested with strain TA 1535 only, 1-MPC did not increase revertant counts above background levels at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See Table 2 for mean summary revertant counts.

## III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. Five strains of *Salmonella typhimurium* were used with and without metabolic activation over a wide exposure range with the highest practicable concentration at the upper end of the range. Positive controls were appropriate for the strain and activation system status producing revertant frequencies within expected ranges. Toxicity does not seem to have been a problem in this study although it was not addressed directly in the report. The results of this study indicate that 1-methylcyclopropene does not cause reverse mutations in *Salmonella typhimurium* under the conditions tested.

### B. Deficiencies

1. Although it is not a major discrepancy, the report did not describe how positive controls were handled (presumably they weren't put into bags).

2. Toxicity should have been addressed more directly (e.g., as in a discussion of range finding studies where background lawns in the petri dishes were examined and observations noted). From the summary tables reporting revertant numbers, it is apparent that revertants were approximately similar to the negative control values. Thus, no toxicity could have been occurring or the numbers

would have been less at the higher exposure levels. The fact that toxicity is not addressed directly in the report should not impact the integrity of this study or the conclusions drawn.

3. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for structurally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

**Table 1**  
**Definitive Assay Revert Count Summaries**

		Mean revertant counts (+/- standard deviation)				
	S-9	Strain TA98	Strain TA100	Strain TA102	Strain TA1535	Strain TA1537
<b>Solvent Controls</b>						
Cyclodextrin	+	30 (+/- 8)	111 (+/- 14)	281 (+/- 15)	28 (+/- 10)	17 (+/- 5)
Cyclodextrin	-	24 (+/- 5)	123 (+/- 10)	249 (+/- 15)	21 (+/- 4)	8 (+/- 2)
<b>Positive Controls</b>						
2-Anthramine	+	1470* (+/- 70)	1182* (+/- 48)	1250* (+/- 58)	315* (+/- 13)	423* (+/- 44)
2-Nitrofluorene	-	683* (+/- 31)				
Sodium azide	-		498* (+/- 15)		585* (+/- 50)	
Mitomycin-C	-			1035* (+/- 25)		
9-Aminoacridine	-					721* (+/- 162)
<b>Exposure Levels</b>						
1000 ppm	+	28 (+/- 9)	137 (+/- 11)	259 (+/- 17)	22 (+/- 2)	16 (+/- 3)
300 ppm	+	37 (+/- 3)	109 (+/- 18)	266 (+/- 14)	26 (+/- 8)	16 (+/- 2)
100 ppm	+	38 (+/- 7)	110 (+/- 13)	287 (+/- 34)	19 (+/- 4)	17 (+/- 3)
1000 ppm	-	20 (+/- 2)	115 (+/- 11)	252 (+/- 10)	23 (+/- 5)	11 (+/- 3)
300 ppm	-	21 (+/- 0)	127 (+/- 15)	245 (+/- 22)	27 (+/- 9)	10 (+/- 1)
100 ppm	-	23 (+/- 6)	134 (+/- 13)	233 (+/- 20)	23 (+/- 6)	9 (+/- 4)

\* Positive response (more than 2 times background).

**Table 2**  
**Confirmatory Assay Revert Count Summaries**

		Mean revertant counts (+/- standard deviation)					Repeat
		Strain	Strain	Strain	Strain	Strain	Strain
	S-9	TA98	TA100	TA102	TA1535	TA1537	TA1535
Solvent Controls							
Cyclodextrin	+	32 (+/- 6)	143 (+/- 14)	211 (+/- 14)	14 (+/- 3)	14 (+/- 4)	18 (+/- 4)
Cyclodextrin	-	27 (+/- 7)	128 (+/- 18)	196 (+/- 5)	10 (+/- 2)	11 (+/- 3)	16 (+/- 5)
Positive Controls							
2-Anthramine	+	1178* (+/- 54)	1082* (+/- 68)	1388* (+/- 46)	286* (+/- 67)	387* (+/- 35)	210* (+/- 23)
2-Nitrofluorene	-	645* (+/- 34)					
Sodium azide	-		474* (+/- 16)		414* (+/- 24)		550* (+/- 12)
Mitomycin-C	-			936* (+/- 30)			
9-Aminoacridine	-					484* (+/- 163)	
Exposure Levels							
1000 ppm	+	32 (+/- 4)	178 (+/- 46)	236 (+/- 19)	28* (+/- 6)	12 (+/- 4)	22 (+/- 5)
500 ppm	+	34 (+/- 6)	151 (+/- 15)	261 (+/- 5)	19 (+/- 9)	11 (+/- 3)	25 (+/- 10)
50 ppm	+	43 (+/- 3)	199 (+/- 17)	233 (+/- 10)	14 (+/- 3)	14 (+/- 2)	26 (+/- 4)
10 ppm	+	38 (+/- 5)	191 (+/- 7)	249 (+/- 3)	19 (+/- 6)	10 (+/- 4)	22 (+/- 2)
1000 ppm	-	24 (+/- 3)	176 (+/- 10)	207 (+/- 4)	19 (+/- 4)	11 (+/- 3)	14 (+/- 12)
500 ppm	-	25 (+/- 8)	158 (+/- 16)	202 (+/- 15)	12 (+/- 3)	9 (+/- 2)	20 (+/- 2)
50 ppm	-	27 (+/- 2)	153 (+/- 22)	199 (+/- 13)	13 (+/- 2)	8 (+/-1)	16 (+/- 2)
10 ppm	-	30 (+/- 3)	152 (+/- 8)	222 (+/- 9)	12 (+/- 6)	6 (+/-3)	25 (+/- 3)

\* Positive response (more than 2 times background).

## DATA EVALUATION RECORD

### 1-METHYLCYCLOPROPENE

STUDY TYPE: IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST  
WITH HUMAN LYMPHOCYTES

Prepared for

Biopesticides and Pollution Prevention Division  
Office of Pesticides Programs  
U.S. Environmental Protection Agency  
Crystal Station I  
2800 Jefferson Davis Highway  
Arlington, VA 22202

Prepared by

Tetrahedron, Inc.  
1414 Key Highway  
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Primary Reviewer:  
Steven T. Cragg, PhD, DABT

Signature: Steven T. Cragg  
Date: 3-July-2001

Secondary Reviewer  
Nasin Begin, PhD

Signature: Nasin Begum  
Date: 7/3/01

Quality Assurance:  
Waqi Alum, PhD.

Signature: W. Alum  
Date: July 3, 2001

EPA Reviewer: Biochemists [Signature] Date 1/29/2002  
Review Section Toxicology Branch (7500C)  
EPA Secondary Reviewer: \_\_\_\_\_ Date \_\_\_\_\_  
Review Section Toxicology Branch (7500C)  
Biochemists

Data Evaluation Record

STUDY TYPE: *In vitro* Mammalian Chromosome Aberration Test with Human Lymphocytes  
OPPTS 870.5375 [§84-2]; OECD Guideline 473 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-04		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Murli, H.M., (2001). 1-Methylcyclopropene Vapor Released from 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.): Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes. Rohm and Haas Company Sponsored Study. Report No. 00RC-194, March 14, 2001. Conducted at Covance Laboratories, Covance Study No. 21917-0-449OECD, MRID 453803-04. Unpublished.

SPONSOR: Rohm and Haas Company  
Toxicology Department  
727 Norristown Road  
Spring House, PA 19477-0904

**EXECUTIVE SUMMARY:** In an initial *in vitro* mammalian chromosome aberration test (MRID 453803-04), cultured human lymphocytes were exposed for approximately 4 hours to atmospheres containing 1-methylcyclopropene (1-MCP) released from a 1-MCP/alpha-cyclodextrin complex (a.i. 3.3%) at nominal concentrations of 0, 100, 300, and 1,000 ppm and harvested approximately 22 hours after initiation of treatment. Replicate cultures of lymphocytes were exposed to 1-MCP with and without an Aroclor-induced rat liver S-9 fraction to determine the influence of a mammalian metabolic activation system upon aberration frequencies. At the end of the incubation period, cells were collected, stained, and mounted on slides where one hundred cells, if possible, per replicate were scored for chromosome aberrations, polyploidy, and endoreduplication. 1-MCP was not tested at higher concentrations than 1,000 ppm because, according to the authors, higher concentrations might result in an explosion hazard. Results of this initial assay indicated no increase in chromosome aberrations, polyploidy, or endoreduplication at any exposure concentration. For lymphocytes not incubated with S-9 fraction, slight cytotoxicity may have been present at 100 and 1000 ppm (but not 300 ppm) as evidenced by reduced mitotic indices of 14% and 8%, respectively. The positive control compounds, mitomycin C (without S-9) and cyclophosphamide (with S-9), produced positive results in expected ranges.

Subsequent to the initial assay, a confirmatory assay was conducted using the same exposure concentrations and procedures. However, lymphocytes without an activation system were exposed to 1-MCP vapors for 19 instead of 4 hours as in the initial assay (lymphocytes with an activation system were exposed again for 4 hours in this subsequent confirmatory assay). Although not stated explicitly in the report, the reason for increasing the exposure time in the confirmatory assay from 4 to 19 hours for lymphocyte cultures not having an S-9 metabolic activation system, apparently arose from the fact that the low and high (but not the mid) exposure concentrations had slightly reduced mitotic indices, suggesting possible cytotoxicity. Mitotic indices again were reduced for lymphocytes incubated without S-9 fraction at the low and high (but not mid) 1-MCP exposure concentrations, 45% and 5%, respectively. No increases

were found at any exposure concentrations in chromosome aberrations, polyploidy, or endoreduplication. The positive control compounds, mitomycin C (without S-9) and cyclophosphamide (with S-9), yielded the expected increases, indicating that the test cells were responsive.

Chemical analysis of exposure atmospheres indicated that actual concentrations ranged from 64% to 116% of target concentrations. The cultured cells reacted appropriately to positive control chemicals, yielding revertant frequencies within expected ranges. The results of this study indicate that 1-methylcyclopropene is not clastogenic in this test system.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for *in vitro* mammalian chromosome aberration data (OPPTS 870.5375).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

## I. MATERIALS AND METHODS

### A Materials:

#### 1. Test Material: 1-Methylcyclopropene

Description: Colorless gas released from *alpha*-cyclodextrin matrix (3.3% a.i.)  
 Lot/Batch #: Lot No. BAS 5-80 (white solid with business confidential composition).  
 Purity: 3.3% within matrix; varying percentages when allowed to vaporize from matrix in Tedlar exposure bags upon addition of water.  
 CAS #: 3100-04-7 (a.i.).  
 Test Article Analysis: Analytical report attached to this report as "APPENDIX II - ANALYSIS OF 1-METHYLCYCLOPROPENE VAPOR CONCENTRATIONS" (pages 54-66).

#### 2. Control Materials:

Negative:  
*alpha*-Cyclodextrin powder without 1-methylcyclopropene (with water added to simulate releasing conditions)

#### Positive:

##### Nonactivation:

Mytomycin C	0.750, 1.00, & 1.50	µg/ml (initial test - ~3 hr)
Mytomycin C	0.150, 0.200, & 0.300	µg/ml (confirmatory test - 19 hr)

##### Activation

Cyclophosphamide	20, 30, & 50	µg/ml
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#### 3. Activation: S9 derived from

<input checked="" type="checkbox"/> Aroclor 1254	<input checked="" type="checkbox"/> Induced	<input checked="" type="checkbox"/> Rat	<input checked="" type="checkbox"/> Liver
<input type="checkbox"/> Phenobarbital	<input type="checkbox"/> Non-induced	<input type="checkbox"/> Mouse	<input type="checkbox"/> Lung
<input type="checkbox"/> None		<input type="checkbox"/> Hamster	<input type="checkbox"/> Other
<input type="checkbox"/> Other			<input type="checkbox"/> Other

S-9 mix was purchased from Molecular Toxicology, Inc. [Moltox], Lot No. 1049 and consisted of the following:

<u>Component</u>	<u>Final Concentration</u>
Nicotinamide-adenine dinucleotide phosphate (NADP)	1.5 mg/ml (1.8 mM)
Isocitric acid	2.7 mg/ml (10.5 mM)
Liver homogenate (S-9) from Aroclor 1254 treated rats	15.0 µg/ml (1.5%)

4. Test Organisms: Human peripheral lymphocytes obtained from health volunteers (i.e., adult non-smokers with no history of radiotherapy, chemotherapy, or drug usage and no current viral infections). To induce division, cells were stimulated with phytohemagglutinin. At predetermined intervals after exposure to the test compound, cells were treated with colchicine to arrest them in metaphase for counting.

Properly maintained? Y

Cell line or strain periodically checked for Mycoplasma contamination? Not applicable.

Cell line or strain periodically checked for karyotype stability? Not applicable.

5. Test compound atmospheric concentrations used

Initial assay (~4 hr exposure [+/- S9] to 1-MCP; harvest at ~22 hr):

Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)

Activated conditions: 0, 100, 300, 1000 ppm (nominal)

Confirmatory assay (~19 hr exposure [-S9], ~4 hr exposure [+S9] to 1-MCP; harvest at ~22 hr):

Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)

Activated conditions: 0, 100, 300, 1000 ppm (nominal)

Duplicate cultures were used for each treatment condition.

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay:

Part of the cytogenetic assay. Mitotic index was used to evaluate cytotoxicity.

2. Cytogenetic assay protocol:

Atmosphere generation: To achieve the desired atmospheric concentration, 1-MCP was released from a quantity of alpha-cyclodextrin matrix selected to achieve a predetermined concentration inside a 12 liter Tedlar air-tight bag. 1-MCP was released when the *alpha*-cyclodextrin matrix containing it was dissolved with warm water inside the bag. Minimal agar dishes (in duplicate) overlaid with the human lymphocytes (with and without metabolic activation systems) were placed inside the bags prior to release of 1-MCP. Once water was mixed with the 1-MCP-containing *alpha*-cyclodextrin matrix, bags were sealed and placed into incubators at 37°C for 24 hours. The atmospheres inside the bags were sampled at 1, 4 and 24 hours by gas chromatography (with a flame ionization detector). Cell cultures were exposed to nominal concentrations of 0, 100, 300, and 1000 ppm.

Initial assay: Peripheral venous blood (0.6 ml) collected from healthy human volunteers was mixed in 15 ml heparinized tubes with 9.6 ml of culture medium consisting of RPMI 1640 supplemented with 15% fetal bovine serum (FBS), penicillin, (100 units/ml), streptomycin (100 ul/ml), L-glutamine (2 mM), and 2% phytohemagglutinin M (PHA-M). This mixture was incubated for two days prior to exposure to facilitate cell division. For the non-activation portion

(-S9) of the assay, the cell cultures were transferred to petri dishes and exposed to the test atmospheres described above for approximately 4 hours. After exposure, cells were washed with buffered saline, refed with RPMI 1640 medium, transferred to 15 ml centrifuge tubes and incubated for the remaining ~18 hr culture period until harvest. Approximately 2 hours prior to harvest, colchicine (Colcemid®) was added to arrest cells in metaphase. For the activation portion of the assay, cell cultures were treated identically to the non-activation samples except that during exposure to 1-MCP, cell medium contained S-9 fraction but not fetal bovine serum (FBS). Cells were harvested after ~22 hours total incubation by centrifuging the cells, discarding the supernatant, swelling the cells with 75 mM KCl hypotonic solution, and then fixing the cells in methanol:acetic acid (3:1 v/v). Cells were then mounted on slides and stained with 5% Geimsa. Slides were coded and subsequently read for mitotic index and chromosomal aberrations.

Confirmatory assay: The confirmatory assay was conducted identically to the initial assay except that cells without S-9 activation systems were exposed to the various concentrations of 1-MCP for 19 instead of 4 hours. This was done to further explore a finding in the initial assay consisting of a slight reduction in mitotic indices at the low and high exposure levels, suggesting cytotoxicity. Cells with S-9 activation were, as in the initial assay, exposed for ~4 hours.

Protocol specifics:

a. Cell Treatment

Cells were exposed to 1-MCP for ~ 4 hours in the initial assay (+/- S9) and, in the confirmatory assay, ~4 hours (+S9) and ~19 hours (-S9).

b. Spindle inhibition

Inhibitor used/concentration: colchicine (Colcemid®)/0.1 µl/ml

Administration time: approximately 2 hours prior to harvest

c. Cell harvest

Cells were harvested after 22 hours total incubation for both the initial and confirmatory assays.

d. Details of slide preparation

See protocol description above.

e. Metaphase analysis

No. cells examined per dose:	100 per culture (in duplicate)
Scored for structural:	Yes
Scored for numerical:	No
Coded prior to analysis:	Yes

f. Evaluation criteria

% cells with structural aberrations  
% cells with more than one structural aberration

evidence for dose-response

Doses with <50% reduction in mitotic index not read (none in this study)

g. Statistical analysis

Fisher's exact test was used to compare exposed cells to negative controls.

Cochran-Armitage was used for linear trend.

## II. REPORTED RESULTS

### A. Initial assay (0, 100, 300, and 1000 ppm) (4hr exposure to 1-MCP; 22 hr total incubation)

No increase in chromosomal aberrations was found at any exposure concentration. For cells not incubated with an S-9 activation system, mitotic indices in the low and high exposure groups were slightly reduced (14% and 8%, respectively). The reductions in mitotic indices were slight and no dose-response for toxicity was evident. To further explore possible cytotoxicity, in the confirmatory assay, cells incubated without S-9 were exposed to 1-MCP for 19 hours (+S-9 cultures were, again, exposed for 4 hours). See the attached tables for individual culture and average results.

### B. Confirmatory assay (0, 10, 50, 500, 1000 ppm) (4 hr [+S9] & 19 hr [-S9] exposure to 1-MCP; 22 hr total incubation)

No increase in chromosomal aberrations was found at any exposure concentration. For cells not incubated with an S-9 activation system, mitotic indices in the low and high exposure groups again were reduced (45% and 5%, respectively). While the low dose mitotic index was reduced more than in the initial assay, a dose-response for cytotoxicity still was not found as the high dose had a higher mitotic index than the low dose and the mid dose was comparable to controls. See the attached tables for individual culture and average results.

## III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. Cultured human lymphocytes were exposed to 1-MCP with and without metabolic activation over a wide exposure range with the highest practicable concentration at the upper end of the range. Positive controls were appropriate for activation system status producing aberration frequencies in the expected ranges. Toxicity does not seem to have been a problem in this study. The results of this study indicate that 1-methylcyclopropene does not cause chromosomal aberrations in cultured human lymphocytes under the conditions tested.

### B. Deficiencies

1. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for structurally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

